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Resistance to Aflatoxin Contamination in Corn as Influenced by Relative Humidity and Kernel Germination

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ABSTRACT

Kernels of corn population GT-MAS:gk, resistant to aflatoxin B_1 production by *Aspergillus flavus*, and susceptible Pioneer hybrid 3154 were tested for aflatoxin when incubated under different relative humidities (RH). High aflatoxin levels were not detected in either genotype at RH < 91%. Resistance in GT-MAS:gk was consistent across all RH levels (91 to 100%) at which significant aflatoxin accumulation was detected. Aflatoxin levels in GT-MAS:gk averaged about 98% less than those in susceptible Pioneer 3154, which suggests that storage of this or other genotypes with similar resistance mechanisms may be possible under moisture conditions less exacting than are required with susceptible hybrids. Results for fungus growth and sporulation ratings on kernel surfaces were similar to those for aflatoxin levels. When kernels of both genotypes were preincubated 3 days at 100% RH prior to inoculation with *A. flavus*, germination percentages increased to very high levels compared to those of kernels that were not preincubated. In preincubated kernels aflatoxin levels remained consistently low in GT-MAS:gk but decreased markedly (61%) in Pioneer 3154. When eight susceptible hybrids were evaluated for aflatoxin accumulation in preincubated kernels, seven of these supported significantly lower toxin levels than kernels not subjected to preincubation. Average reduction across hybrids was 83%, and reductions within hybrids ranged from 68 to 96%. Preincubated kernels of one susceptible hybrid (Deltapine G-4666) supported aflatoxin levels comparable to those in resistant GT-MAS:gk. Data suggest that an inhibitor of aflatoxin biosynthesis may be induced during kernel germination. Possible mechanisms for embryo effects on resistance to aflatoxin accumulation are discussed.

Key words: *Aspergillus flavus*, food safety, induced resistance, inhibitor, maize, mycotoxin, *Zea mays*

Aspergillus flavus Link has been studied intensively since 1960, when aflatoxins were identified as causal agents of a poultry disease *(19, 26)*. Aflatoxins later were recognized as a potential human health hazard *(13)*. These mycotoxins are extremely potent, naturally occurring carcinogens that occur in food for livestock and humans *(7, 16, 30)*. Corn (*Zea mays* L.) is an important crop in the grain and livestock economy of the United States and worldwide. Grain colonization by *A. flavus* and subsequent aflatoxin contamination are chronic problems in the southeastern United States and occur periodically in the midwest *(12)*. Although *A. flavus* had been observed as an ear mold *(31)*, it was thought to occur only in storage until it was found growing and producing aflatoxins in developing corn ears in the field *(1)*.

Corn genotypes now are routinely screened for resistance to aflatoxin production as part of a long-term approach to eliminate preharvest contamination by aflatoxins *(8, 15, 35)*. Both field and laboratory studies identified corn population GT-MAS:gk as a promising source of aflatoxin resistance *(4, 15, 35)*. However, aflatoxin contamination still can be a significant problem in storage. Resistant corn genotypes will be most useful if their resistance is expressed under both preharvest and postharvest storage conditions. The stability of resistance in GT-MAS:gk under different storage conditions has not been examined.

Preharvest aflatoxin resistance in GT-MAS:gk was associated with cutin and wax in the pericarp and with other preformed compound(s) within kernels *(15)*. Brown et al. *(4)* showed further that a living embryo was essential for expression of resistance in this genotype. Killing the embryo using any of several techniques resulted in aflatoxin levels that were significantly higher than those in nonwounded kernels of GT-MAS:gk as well as those in susceptible hybrids. That the viable embryo exerts such profound control over resistance in GT-MAS:gk suggests that metabolic processes, perhaps protein synthesis *(5, 6, 9)*, turned on during germination may be important for influencing aflatoxin production in other corn genotypes as well.

The objectives of our research were (i) to determine the effects of relative humidity on aflatoxin production in resistant corn genotype GT-MAS:gk infected with *A. flavus*, and (ii) to study the effect of germination on aflatoxin production in resistant and susceptible corn genotypes. A preliminary report has been published *(14)*.

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MATERIALS AND METHODS

General methods

Studies utilized resistant corn population GT-MAS:gk *(4, 15)* and commercial corn hybrids Asgrow RX 899, Dekalb 689, Deltapine G-4666, McCurdy 7477, ORO 188 and 200W, and Pioneer 3154 and 3165. In previous studies, these hybrids exhibited a range in susceptibility to aflatoxin contamination by *A. flavus (15).* Kernels were free of insecticides, fungicides, and dyes normally used to treat seed. Kernels were surface-sterilized by immersing in 0.75% NaOCl for 5 min and rinsing in three changes of sterile deionized water. This was done to rid kernel surfaces of microorganisms which may reduce aflatoxin production by *A. flavus.* Strain AF13 of *A. flavus,* which produces large quantities of aflatoxins *(3, 10),* was used in all studies. The fungus was grown on V8 juice agar (5% V8 juice, 2% agar) under standard conditions (30°C in darkness). Fungus inoculum consisted of conidia from 7-day-old cultures suspended in sterile deionized water. The fungus was maintained as agar discs (3 mm in diameter) from 4-day-old cultures in sterile deionized water at 4°C. Selected relative humidities (RH) for corn kernel incubation were obtained using saturated salt solutions *(11, 34, 36).* Salts and corresponding RH values were $Na_2Cr_2O_7 \cdot H_2O$ (52.5%); $NaNO_2$ (63%); $NaNO_3$ (72.5%); NH_4Cl (77.5%); $(NH_4)_2SO_4$ (84.5%); KNO_3 (91%); $K_2Cr_2O_7$ (97.5%). Deionized water was used for 100% RH.

Corn kernel inoculation and incubation

Effects of RH on expression of resistance to *A. flavus* growth and aflatoxin production were tested in two experiments using resistant GT-MAS:gk and susceptible Pioneer 3154. In experiment 1, dry kernels of both genotypes were immersed in a suspension of *A. flavus* conidia (10^6/ml), removed immediately, and allowed to dry at room temperature. Five kernels of each genotype, which constituted one experimental unit, were placed in a single cell (35 mm in diameter, 20 mm in height) of a 6-cell culture dish (Costar, Cambridge, MA). Dishes were sealed in plastic containers (180 by 150 by 90 mm) (Nalge Company, Rochester, NY) that contained 150 ml of water or saturated salt solution. Kernels did not contact water or salt solutions directly. All treatments were replicated 12 times. Kernels were incubated as described at different RH for 7 days, and then dried at 60°C for 2 days to stop further fungus growth and toxin synthesis. This experiment was conducted twice.

In experiment 2, dry kernels of both genotypes were incubated as described above for 3 days over water or saturated salt solution prior to inoculation with *A. flavus.* This preincubation treatment allowed time for kernel moisture to equilibrate with ambient RH prior to inoculation. Following inoculation, kernels were returned to the same environments and incubated for 7 additional days. All treatments were replicated 12 times. Kernels were dried as described. This experiment was conducted three times.

Kernels of resistant GT-MAS:gk and all eight susceptible hybrids were preincubated 0 or 3 days at 100% RH. Kernels were inoculated with *A. flavus* as described and incubated 7 additional days at 100% RH. All treatments were replicated 12 times, and kernels were dried as described. This experiment was conducted twice.

Fungal sporulation rating and aflatoxin analysis

Fungal sporulation, an index of fungal growth, on individual kernels was rated at the end of each test according to the following scale: 0, mycelium visible on kernel surface but no sporulation; 1, 1 to 20%; 2, 21 to 40%; 3, 41 to 60%; 4, 61 to 80%, and 5, 81 to 100% of the kernel surface covered by conidiophores bearing conidia.

Levels of aflatoxin B_1 were determined using official methods of the American Oil Chemists Society *(2)* with modifications *(4, 15).* Five-kernel experimental units were powdered, weighed, and combined with methylene chloride (30 ml) in 50-ml flasks. After shaking (60 rpm) for 30 min, the methylene chloride and kernel powder were filtered through Whatman No. 1 paper (Whatman International Ltd., Maidstone, England) into 50-ml beakers and evaporated to dryness. The beakers were rinsed with methylene chloride, which was transferred to 8-ml vials and evaporated to dryness. Vial residues were dissolved in 2 ml of benzene: acetonitrile (98:2), spotted on thin-layer chromatography plates (Silica Gel 60 F_{254}) (EM SCIENCE, Gibbstown, NJ), and developed in anhydride ether:methanol:water (96:3:1). Levels of aflatoxin B_1 were quantified directly on plates using a scanning densitometer with a fluorometry attachment (Model CS-930, Shimadzu Scientific Instruments, Inc., Tokyo, Japan). A commercial aflatoxin B and G mixture (Sigma Chemical Company, St. Louis, MO) served as a standard.

Statistical analysis

Aflatoxin B_1 data were transformed [log (X + 1)] prior to analysis to equalize variances. All data were analyzed using the analysis of variance procedure of the Statistical Analysis System (SAS) *(28, 29).* When the number of treatment levels exceeded two, differences between levels were tested using least significant difference (LSD).

RESULTS

Relative humidity effects on resistance to aflatoxin production

Aflatoxin was not detected in kernels incubated at <80% RH, and minimal amounts of toxin were detected when kernels were incubated at 80 and 84.5% RH (Table 1).

TABLE 1. *Aflatoxin B_1 levels and fungal sporulation ratings on corn kernels of two genotypes inoculated with* Aspergillus flavus *and then incubated at different relative humidity levels for 7 days at 30°C*

Relative humidity (%)	Aflatoxin B_1 (ppb)[a,b]		Sporulation rating[a,c]	
	Corn genotype		Corn genotype	
	Pioneer 3154	GT-MAS:gk	Pioneer 3154	GT-MAS:gk
72.5	nd[d]	nd	nd	nd
77.5	nd	nd	0 B	nd
80.0	15 B	4 B	0 B	nd
84.5	20 B	7 B	0 B	0 B
91.0	2,215 A	45 A	2.0 A	0.4 A
97.5	3,758 A	68 A	2.3 A	0.4 A
100.0	3,473 A	79 A	2.4 A	0.5 A

[a] Combined means from two tests are presented. Each mean was obtained from 24 observations. Within columns, means followed by the same letter did not differ significantly ($P > 0.05$) according to LSD.

[b] Analyses were done on transformed [log (X + 1)] data, but the means presented are not transformed.

[c] Fungal sporulation was rated as follows: 0, mycelium visible on kernel surface but no sporulation; 1, 1 to 20%; 2, 21 to 40%; 3, 41 to 60%; 4, 61 to 80%; and 5, 81 to 100% of the kernel surface covered by conidiophores bearing conidia.

[d] nd: Aflatoxin, sporulation, and/or mycelium not detected.

TABLE 2. *Aflatoxin B_1 levels and fungal sporulation ratings on corn kernels of two genotypes preincubated for 3 days at different relative humidity levels as indicated, inoculated with* Aspergillus flavus, *and then incubated at the same relative humidity levels as preincubation for 7 days at 30°C*

Relative humidity (%)[b]	Aflatoxin B_1 (ppb)[a,c]		Sporulation rating[a,d]	
	Corn genotype		Corn genotype	
	Pioneer 3154	GT-MAS:gk	Pioneer 3154	GT-MAS:gk
72.5	nd[e]	nd	nd	nd
77.5	nd	nd	0 D	nd
80.0	6 C	11 B	0 D	0 C
84.5	34 C	9 B	0.1 C	0 C
91.0	5,034 A	108 A	2.8 B	0.5 B
100.0	1,952 B	193 A	4.0 A	2.0 A

[a] Combined means from three experiments are presented. Each mean was obtained from 36 observations. Within columns, means followed by the same letter did not differ significantly ($P > 0.05$) according to LSD.

[b] Relative humidity (%) for both preincubation and incubation.

[c] Analyses were done on transformed [log (X + 1)] data, but the means presented are not transformed.

[d] Fungal sporulation was rated as follows: 0, mycelium visible on kernel surface but no sporulation; 1, 1 to 20%; 2, 21 to 40%; 3, 41 to 60%; 4, 61 to 80%; and 5, 81 to 100% of the kernel surface covered by conidiophores bearing conidia.

[e] nd: Aflatoxin, sporulation, and/or mycelium not detected.

Toxin production was similar between resistant GT-MAS:gk and susceptible Pioneer 3154 at these RH levels. Aflatoxin production increased significantly in both genotypes when kernels were incubated at 91% RH, but did not increase further as RH increased to 100% (Table 1). Resistance in GT-MAS:gk was most evident at these RH levels: toxin levels in GT-MAS:gk were about 98% lower than those in susceptible Pioneer 3154.

Results for sporulation ratings were similar to those for aflatoxin levels. Sporulation was not observed on either genotype until the RH level was ≥91%, and sporulation ratings did not change as the RH increased to 100% (Table 1). Sporulation ratings in GT-MAS:gk were ≥80% lower than those in susceptible Pioneer 3154. Genotypes differed with regard to initial mycelium growth on kernel surfaces. The mycelium of *A. flavus* was visible on surfaces of Pioneer 3154 kernels incubated at 77.5% RH, whereas a mycelium was not observed on GT-MAS:gk kernels at RH levels <84.5% (Table 1).

Preincubation effects on resistance to aflatoxin production

At RH ≤ 91%, kernels of Pioneer 3154 and GT-MAS:gk that were preincubated 3 days prior to inoculation supported aflatoxin levels and *A. flavus* sporulation ratings that were similar to those on kernels that were not preincubated (Tables 1 and 2). Aflatoxin levels were not detected or were very low at RH < 91%, and increased significantly in both genotypes at 91% RH (Table 2). GT-MAS:gk again supported toxin production that was about 98% lower than that in Pioneer 3154. However, minor differences were observed in sporulation ratings for these genotypes. Mycelium was detected on preincubated GT-MAS:g kernels at lower RH than on kernels that were not preincubated, although sporulation still was not observed until the RH level reached 91% (Table 2). On Pioneer 3154, both mycelium growth and sporulation occurred at lower RH levels on preincubated kernels (Table 2).

Kernel germination was very high following 3 days of preincubation at 100% RH. Under these conditions, aflatoxin levels remained consistently low in resistant GT-MAS:gk but decreased markedly (61%) in susceptible Pioneer 3154, relative to toxin levels at 91% RH (Table 2). This is in direct contrast to results from Pioneer 3154 kernels that were not preincubated (Table 1). Sporulation ratings for both genotypes increased significantly on kernels preincu-

TABLE 3. *Kernel germination, aflatoxin B_1 levels, and fungal sporulation ratings of kernels of resistant and susceptible corn genotypes that were not preincubated or preincubated for 3 days at 100% relative humidity, inoculated with* Aspergillus flavus, *and then incubated at 100% relative humidity for 7 days at 30°C*

Corn genotype	Kernel germination (%)[a]		Aflatoxin B_1 (ppb)[a,b]			Sporulation rating[a,c]		
	Not preincubated	Preincubated 3 days	Not preincubated		Preincubated 3 days	Not preincubated		Preincubated 3 days
GT-MAS:gk	4.2	98.3	52		97	0.6	*	1.7
Asgrow RX 899	17.5	93.0	7,398	*[d]	2,008	2.2	*	3.1
Dekalb 689	3.0	100.0	7,098	*	928	2.0	*	2.8
Deltapine G-4666	16.6	100.0	1,793	*	101	2.3		2.4
McCurdy 7477	75.8	100.0	1,906		1,204	2.4		2.6
Oro 188	12.5	94.2	9,304	*	1,489	1.8	*	2.3
Oro 200W	6.6	100.0	4,165	*	1,325	1.6	*	2.3
Pioneer hybrid 3154	8.3	97.5	6339	*	262	3.6	*	4.1
Pioneer hybrid 3165	15.8	98.3	14,396	*	2,947	2.6		2.9

[a] Combined means from two experiments are presented. Each mean was obtained from 24 observations.

[b] Analyses were done on transformed [log (X + 1)] data, but the means presented are not transformed.

[c] Fungal sporulation was rated as follows: 0, mycelium visible on kernel surface but no sporulation; 1, 1 to 20%; 2, 21 to 40%; 3, 41 to 60%; 4, 61 to 80%; and 5, 81 to 100% of the kernel surface covered by conidiophores bearing conidia.

[d] For each corn genotype, significant ($P \leq 0.05$) differences between treatments for each variable are indicated with an asterisk.

bated at 100% RH, relative to sporulation ratings obtained at lower RH levels (Table 2).

Germination effects on resistance to aflatoxin production

Preliminary results indicated that, at 100% RH, germination (radicle visible) was much greater if kernels were subjected to a 3-day preincubation prior to the standard 7-day incubation, but not if this preincubation period were omitted. Several recent studies indicated that defense mechanisms against fungus infection may be induced during the germination process in corn *(5, 6, 9, 14)*. Therefore, experiment 3 evaluated the effects of preincubation and subsequent kernel germination on growth and toxin production by *A. flavus* and on the expression of resistance in corn genotypes. Kernels of resistant GT-MAS:gk that were preincubated for 3 days showed 98.3% germination, whereas only 4.2% of kernels not preincubated germinated following the standard 7-day incubation period (Table 3). Aflatoxin levels in preincubated GT-MAS:gk kernels were similar to those in kernels not preincubated, but preincubated kernels had significantly higher sporulation ratings (Table 3). These results are consistent with data from this genotype in Table 2.

For most (7 of 8) susceptible hybrids tested, preincubated kernels germinated readily (≥93%), whereas those not preincubated had lower germination (≤17.5%) after the standard 7-day incubation (Table 3). Aflatoxin levels in preincubated kernels of these hybrids were significantly lower than those in kernels not preincubated (Table 3). Across these seven hybrids, reductions in aflatoxin levels due to preincubation averaged about 83% and ranged from 68 to 96%. The exception to this was McCurdy 7477, which germinated readily regardless of preincubation and showed no difference in aflatoxin levels (Table 3). Sporulation ratings were higher on preincubated kernels, but not for all genotypes (Table 3). The hybrids Deltapine G-4666, McCurdy 7747, and Pioneer 3165 showed no increase in sporulation following preincubation.

DISCUSSION

The development of *A. flavus* and subsequent production of aflatoxin in corn are controlled by many factors, among which relative humidity is very important *(27)*. In the present study, aflatoxin levels were very low when kernels of GT-MAS:gk and Pioneer 3154 were incubated at 80 and 84.5% RH, but reached typical levels at RH ≥ 91%. These data agree closely with those of Lillehoj *(20)*, who reported significant aflatoxin production when kernels were at RH ≥ 87%, but limited toxin production at lower RH values. Previously, we showed *(15, 32)* that GT-MAS:gk kernels contain significantly more pericarp wax than do Pioneer 3154 kernels, and that resistance in GT-MAS:gk is due in part to increased amounts of wax. Therefore we hypothesized that thicker pericarp wax layers may allow GT-MAS:gk, or genotypes with similar resistance mechanisms, to be subjected to higher moisture levels in storage and still resist infection and toxin production. Our results show that resistance in GT-MAS:gk was stable across a range of RH levels, and that aflatoxin levels still were about 98% lower than in susceptible Pioneer 3154 even under conditions optimal for toxin production.

Mycelium growth and sporulation on kernels of both genotypes mirrored aflatoxin production at RH ≥ 80%; that is, high toxin levels generally were detected when *A. flavus* sporulation was abundant on kernel surfaces. Limited mycelium growth was detected consistently on Pioneer 3154 kernels at 77.5% RH, which is lower than required for aflatoxin production according to our results and those of others *(20)*. This suggests that aflatoxin synthesis in corn kernels may be more sensitive to moisture levels than are normal fungal growth processes. A similar conclusion was reached by Northolt and colleagues *(24, 25)*, who used artificial media to study effects of water availability on *A. flavus* growth and aflatoxin production.

Aflatoxin levels in most susceptible hybrids were markedly lower in preincubated kernels, which were characterized by high germination percentages, than in kernels that were not preincubated. In fact, aflatoxin levels in germinated kernels of one hybrid (Deltapine G-4666) were comparable to those in resistant GT-MAS:gk. Keller et al. *(18)* dissected corn kernels, removed different tissues, and inoculated these tissues with *A. flavus, A. nidulans,* and *A. parasiticus*. They found that germinating embryos were resistant to growth and toxin production by all three *Aspergillus* species. Lindsey and Turner *(21)* reported compounds in immature peanut embryos that were inhibitory to *A. flavus* growth in culture. Brown et al. *(4)* showed that resistance to *A. flavus* in GT-MAS:gk kernels is associated with a living embryo. When examined together, the results from current and previous studies suggest that compound(s) are produced in germinating corn kernels that greatly reduce aflatoxin production by *A. flavus*. Because of our experimental design, we were unable to determine whether these compounds were generally present in germinating kernels or were induced by *A. flavus* infection. In related studies *(5, 6, 9)*, induced proteins were present in high levels only in germinating corn kernels, and their levels increased even further when kernels were infected by the fungus *Fusarium moniliforme*. It was not reported, however, whether these induced proteins were antifungal.

The mechanism responsible for reduced aflatoxin synthesis in germinating kernels of susceptible hybrids is unclear, but it may be similar to that in GT-MAS:gk. Guo et al. *(15)* found that, in addition to pericarp wax and cutin, resistance in this genotype may be due to preformed compounds within kernels. Our hypothesis is that these compounds are absent, or present at low levels, in dry kernels of susceptible hybrids, and that their levels increase during germination. Several researchers have reported that corn kernels contain several proteins with antifungal activity. Huynh et al. *(17)* identified a 22-kDa protein that inhibited the growth of *Fusarium oxysporum* and *Alternaria solani*. Another protein of similar size, zeamatin, inhibited the growth of *Candida albicans (33)*. Neucere and Zeringue *(23)* showed that kernel proteins from 'Yellow Creole' (resistant to aflatoxin contamination) reduced the growth of *A. flavus*, whereas the fungus was not affected by kernel

proteins from the susceptible 'Huffman.' Nagarajan and Bhat *(22)* showed high levels of a low-molecular-weight protein in corn hybrid 'Opaque-2,' which is resistant to aflatoxin contamination. In peanuts, resistance to *A. flavus* in freshly harvested seed was associated with several compounds, possibly phenolics, that were extracted from cotyledons *(21)*.

McCurdy 7477 was the only susceptible corn hybrid in our studies that did not support reduced aflatoxin levels in preincubated kernels. This can be explained by examining germination values at 100% RH without preincubation (Table 3). Kernels of this hybrid showed nearly 76% germination under these conditions, which was more than fourfold greater than any other corn genotype tested. Consequently, comparisons of toxin levels in McCurdy 7477 kernels with high and low germination levels were not possible.

Results from our studies show that resistance in GT-MAS:gk was stable regardless of the RH at which the grain was incubated, which suggests that storage of this or related genotypes may be possible under moisture conditions less exacting than are required with susceptible hybrids. Our results also indicate that kernels of normally susceptible hybrids become moderately to highly resistant during germination. This phenomenon was observed in nearly all susceptible hybrids tested. Further research is needed to identify these resistance mechanisms in germinating kernels. Understanding how the viable embryo influences resistance to aflatoxin production may provide future opportunity to modify corn genotypes to support lower toxin levels.

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REFERENCES

1. Anderson, H. W., E. W. Nehring, and W. R. Wichser. 1975. Aflatoxin contamination of corn in the field. J. Agric. Food Chem. 23:775–782.
2. Anonymous. 1988. Aflatoxin in maize, AJ3–87. *In* Official methods and recommended practices. Am. Oil Chem. Soc., Champaign, IL.
3. Brown, R. L., P. J. Cotty, and T. E. Cleveland. 1991. Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. J. Food Prot. 54:623–626.
4. Brown, R. L., P. J. Cotty, T. E. Cleveland, and N. W. Widstrom. 1993. Living maize embryo influences accumulation of aflatoxin in maize kernels. J. Food Prot. 56:967–971.
5. Casacuberta, J. M., P. Puigdoménech, and B. S. Segundo. 1991. A gene coding for a basic pathogenesis-related (PR-like) protein from *Zea mays*. Molecular cloning and induction by a fungus (*Fusarium moniliforme*) in germinating maize seeds. Plant Mol. Biol. 16:527–536.
6. Casacuberta, J. M., D. Raventós, P. Puigdoménech, and B. S. Segundo. 1992. Expression of the gene encoding the PR-like protein PRms in germinating maize embryos. Mol. Gen. Genet. 234:97–104.
7. Cleveland, T. E., and D. Bhatnagar. 1992. Molecular strategies for reducing aflatoxin levels in crops before harvest, p. 205–228. *In* D. Bhatnagar and T. E. Cleveland (eds.), Molecular approaches to improving food quality and safety. Van Nostrand Reinhold, New York.
8. Cleveland, T., J. Cary, R. Brown, T. Jacks, H. Zeringue, J. Neucere, D. Bhatnagar, G. Payne, C. Woloshuk, A. Weissinger, P. Ozias-Akins, S. Tuzun, P. Gay, C. Chlan, B. Guo, and J. Russin. 1993. Control of *Aspergillus flavus* infection and/or aflatoxin biosynthesis using host and non-host genes, p. 45–47. *In* Proceedings of the aflatoxin elimination workshop. Little Rock, AR.
9. Cordero, M. J., D. Raventós, and B. S. Segundo. 1992. Induction of PR protein in germinating maize seeds infected with the fungus *Fusarium moniliforme*. Physiol. Mol. Plant Pathol. 41:189–200.
10. Cotty, P. J. 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 78:808–814.
11. Dhingra, O. D., and J. B. Sinclair. 1985. Basic plant pathology methods. CRC Press, Boca Raton, FL.
12. Diener, U. L., R. L. Asquith, and J. W. Dickens. 1983. Aflatoxin and *Aspergillus flavus* in corn. South. Coop. Ser. Bull. 279. Alabama Agric. Exp. Station, Auburn, AL.
13. Goldblatt, L. A. 1969. Aflatoxin: scientific background, control, and implications. Academic Press, New York.
14. Guo, B. Z., J. S. Russin, R. L. Brown, T. E. Cleveland, and N. W. Widstrom. 1994. Effects of relative humidity and preincubation on growth of *Aspergillus flavus* and aflatoxin production in maize kernels. Phytopathology 84:1064.
15. Guo, B. Z., J. S. Russin, T. E. Cleveland, R. L. Brown, and N. W. Widstrom. 1995. Wax and cutin layers in maize kernels associated with resistance to aflatoxin production by *Aspergillus flavus*. J. Food Prot. 58:296–300.
16. Hsieh, D. P. H. 1989. Potential human health hazards of mycotoxins, p. 69–80. *In* S. Natori, K. Hashimoto, and Y. Ueno (ed.), Mycotoxins and phycotoxins. Elsevier, Amsterdam.
17. Huynh, Q. K., J. R. Borgmeyer, and J. F. Zobel. 1992. Isolation and characterization of a 22 kDa protein with antifungal properties from maize seeds. Biochem. Biophys. Res. Comm. 182:1–5.
18. Keller, N. P., R. A. E. Butchko, B. Sarr, and T. D. Phillips. 1994. A visual pattern of mycotoxin production in maize kernels by *Aspergillus* spp. Phytopathology 84:483–488.
19. Lancaster, M. C., F. P. Jenkins, and J. Philip. 1961. Toxicity associated with certain samples of groundnuts. Nature 192:1095–1096.
20. Lillehoj, E. B. 1983. Effect of environmental and cultural factors on aflatoxin contamination of developing corn kernels, p. 27–34. *In* U. L. Diener, R. L. Asquith, and J. W. Dickens (ed.), Aflatoxin and *Aspergillus flavus* in corn. South. Coop. Ser. Bull. 279, Alabama Agric. Exp. Station, Auburn, AL.
21. Lindsey, D. L., and R. B. Turner. 1975. Inhibition of growth of *Aspergillus flavus* and *Trichoderma viride* by peanut embryos. Mycopathologia 55:149–152.
22. Nagarajan, V., and R. V. Bhat. 1972. Factors responsible for varietal differences in aflatoxin production in maize. J. Agric. Food Chem. 20:911–914.
23. Neucere, J. N., and H. J. Zeringue, Jr. 1987. Inhibition of *Aspergillus flavus* growth by fractions of salt-extracted proteins from maize kernel. J. Agric. Food Chem. 35:806–808.
24. Northolt, M. D., and L. B. Bullerman. 1982. Prevention of mold growth and toxin production through control of environmental conditions. J. Food Prot. 45:519–526.
25. Northolt, M. D., C. A. H. Verhulsdonk, P. S. S. Soentoro, and W. E. Paulsch. 1976. Effect of water activity and temperature on aflatoxin production by *Aspergillus flavus*. J. Milk Food Technol. 39:170–174.
26. Sargeant, K., A. Sheridan, J. O'Kelley, and R. B. A. Carnaghan. 1961. Toxicity associated with certain samples of groundnuts. Nature 192:1096–1097.
27. Sauer, D. B., and J. Tuite. 1987. Conditions that affect growth of *Aspergillus flavus* and production of aflatoxin in stored maize, p. 41–50. *In* M. S. Zuber, E. B. Lillehoj, and B. L. Renfro (ed.), Aflatoxin in maize: a proceedings of the workshop. Centro Internacional de Mejoramiento de Maize y Trigo, Mexico, D.F.
28. Statistical Analysis System (SAS) Institute. 1991. SAS procedures guide, release 6.03 ed. SAS Institute, Cary, NC.
29. Statistical Analysis System (SAS) Institute. 1991. SAS/STAT user's guide, release 6.03 ed. SAS Institute, Cary, NC.
30. Smith, J. E., and M. O. Moss. 1985. Mycotoxins: formation, analyses, and significance. John Wiley & Sons, Chichester, NY.

31. Taubenhaus, J. J. 1920. A study of the black and yellow molds of ear corn. Texas Agric. Exp. Station Bull. 270, College Station, TX.
32. Tubajika, K. M., B. Z. Guo, J. S. Russin, R. L. Brown, T. E. Cleveland, and N. W. Widstrom. 1995. Factors associated with resistance to aflatoxin production in maize. Phytopathology 85:512.
33. Vigers, A. J., W. K. Roberts, and C. P. Selitrennikoff. 1991. A new family of plant antifungal proteins. Mol. Plant-Microbe Int. 4:315–323.
34. Weast, R. C. (ed.). 1975. Handbook of chemistry and physics. CRC Press, Cleveland, OH.
35. Widstrom, N. W., W. W. McMillian, and D. M. Wilson. 1987. Segregation for resistance to aflatoxin contamination among seeds on an ear of hybrid maize. Crop Sci. 27:961–963.
36. Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41:232–237.